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(54) **Process for the manufacture of a vitamin E intermediate**

(57) Trimethylhydroquinone-1-monoacetate can be prepared from trimethylhydroquinone diacetate by a selective enzymatic monosaponification using a lipase.

The activity of the enzymes can be increased by immobilisation on a solid carrier material. When the enzyme is immobilized on an appropriate carrier the monosaponification can be performed continuously instead of

batch-wise.

The trimethylhydroquinone-1-monoacetate can be converted into (all-*rac*)- α -tocopherol or its acetate by reaction with isophytol or an equivalent thereof, either directly or followed, if desired, by acetylation.

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Description

[0001] The major commercial form of vitamin E is its acetate derivative, synthesized by acetylation of (all-*rac*)- α -tocopherol, e.g. with acetic anhydride.

[0002] Industrial syntheses of (all-*rac*)- α -tocopherol are based on the condensation of trimethylhydroquinone (TMHQ) with isophytol, phytol or a derivative thereof, such as a phytol halide. TMHQ is normally obtained from 2,3,6-trimethylphenol which is expensive, however, and acidic catalysts have to be used for the condensation of the TMHQ with isophytol, phytol or a derivative thereof, such as a phytol halide.

[0003] Alternatively, (all-*rac*)- α -tocopherol acetate can be synthesized by condensing trimethylhydroquinone-1-monoacetate (TMHQ-1-MA) with isophytol or an equivalent thereof. The TMHQ-1-MA used in this alternative synthesis can be obtained from the much less expensive α -isophorone via ketoisophorone and trimethylhydroquinone diacetate (TMHQ-DA), the latter having to undergo an absolutely regioselective mono-deacetylation which is difficult to achieve by methods known from literature (e.g. by treatment with aqueous alkaline bases), however.

[0004] It has now been found that TMHQ-DA can be absolutely regioselectively converted into TMHQ-1-MA by subjecting the TMHQ-DA to an enzymatic monosaponification by means of a lipase.

[0005] In a preferred embodiment of the present invention the lipase is immobilised on a solid carrier material. Said carrier material can be a hydrophobic carrier, e.g. a polypropylene carrier such as ACCUREL MP1001, provided by Membrana GmbH, Obernburg (Germany). A carrier of a different nature, namely the alkaline catalyst carrier CELITE [chemical composition: 87% SiO₂, 0.9% CaO, 6.1% Al₂O₃, 1.6% Fe₂O₃, 1.6% Na₂O + K₂O; pH (10% suspension, 25°C) = 8.5] which is often used for the immobilization of enzymes, did not give a satisfying performance of the immobilized enzyme, however.

[0006] Lipases which are suitable for the purposes of the present invention include those belonging to enzyme class EC 3.1.1.3.

[0007] Among the various lipases which are available on the market the following, in particular, have proved efficient for the purposes of the present invention: *Thermomyces lanuginosus* lipase (TLL); *Mucor mihei* lipase (MML); *Alcaligenes* spec. lipase (ASL); *Candida rugosa* lipase (CRL); *Candida antarctica* (fraction B) lipase (CAL(B)); and *Pseudomonas* spec. lipase (PSL), e.g. *Pseudomonas fluorescens* lipase (PFL). Preferred lipases are PSL, PFL and TLL; with TLL being particularly preferred.

[0008] The enzymatic monosaponification of the invention is conveniently carried out in a hydrophobic solvent, e.g. in 1-methyl-2-pyrrolidone or, particularly, in an ether solvent such as tert.-butyl methyl ether, butyl ether, methyl 2-methyl-2-butyl ether or the like with tert.-butyl methyl ether being particularly preferred.

[0009] Conveniently from about 0.01 to about 99.5 vol %, preferably about 0.03 to about 20 vol %, most preferably about 0.09 to about 5 vol % of water or buffer, such as phosphate buffer, may be added to the ether solvent. Ethanol may be present in a concentration of up to 1 %.

[0010] Tetrahedron 56 (2000) 317-321 describes, inter alia, the selective monosaponification of 2-methyl-1,4-diacetoxynaphthalene into the corresponding 1-acetoxy-4-hydroxy compound by means of the free enzyme PSL in tert.-butyl methyl ether in the presence of water. When repeating this experiment over a time up to 185 hours it was found, however, that inconsistent results were obtained. Furthermore, when treating TMHQ-DA with the free enzyme PSL under the same reaction conditions over a time of up to about 300 hours, the initial reaction rate was only about one third. As against that the monosaponification of TMHQ-DA by means of immobilized PSL over a time of <100 hours resulted in an almost quantitative conversion, and similar results were obtained with immobilized PFL and immobilized TLL.

[0011] The reaction rate of the monosaponification of the present invention normally increases with increased reaction temperatures. The maximum temperature is, of course, limited by the boiling point of the solvent (55°C in the case of tert.-butyl methyl ether) but still higher temperatures can be achieved when performing the enzymatic monosaponification under pressure. With respect to some of the lipases, particularly TLL, the temperature may be raised up to about 60 to 80°C.

[0012] The enzymatic monosaponification of the invention is thus conveniently carried out in a temperature range of from about 4 to about 80°C, preferably in the range of from about 20 to about 75°C.

[0013] The ratio of enzyme, both free and immobilized, to the substrate (TMHQ-DA) can vary in a rather broad range, conveniently of from about 0.001 g/g to about 10 g/g, preferably from about 0.01 to about 0.2 g/g.

[0014] The ratio of the substrate (TMHQ-DA) to the solvent can likewise vary in a rather broad range, conveniently of from about 0.001 g/g to about 100 g/g, preferably from about 0.01 g/g to about 0.8 g/g.

[0015] When the enzyme is immobilized on an appropriate carrier the monosaponification of the invention can advantageously be performed continuously, e.g. in a fixed-bed reactor or a continuous stirred tank reactor, instead of batch-wise.

[0016] As mentioned earlier, the TMHQ-1-MA obtained by the enzymatic monosaponification of the invention can be converted into (all-*rac*)- α -tocopheryl acetate, e.g. by reaction with isophytol. If (all-*rac*)- α -tocopherol should be

present in the crude product, such (all-*rac*)- α -tocopherol can, if desired, be converted into its acetate by acetylation, e.g. by means of acetic anhydride.

[0017] The following Examples illustrate the invention in more detail but are not intended to limit its scope in any way.

5 Example 1: Batch experiments in glass vessels using free lipases

[0018] 5 ml of tert.-butyl methyl ether, 50 μ l of water, 1.67 mg free enzyme [lipases are from Fluka Chemie AG (Buchs, Switzerland)] and 80 mg TMHQ-DA [crude, i.e. material which results from rearrangement-aromatization of ketoisophorone and consists of about 90 % TMHQ-DA and about 9 % of trimethylcatechol diacetate (TMC-DA)] are added
10 into a vessel. The headspace of the vessel is flushed with nitrogen. The vessel is placed into an incubator at 50°C and stirred at 700 rpm to ensure good mixing. To take samples, the vessels are opened, 500 μ l are taken out, the headspace is flushed with nitrogen and the vessel is closed again. The sample is then diluted to a substrate or product concentration of 0.5-1.0 wt % and analyzed by GC.

Table 1:

Conversion for various lipases and TLL after 6, 12, 24 or 48 h, respectively (E/S = 1/48, C _{TMHQ-DA} =0.019 g/g)		
	conversion X [%]	
time [h]	PFL	TLL
6	5	5
12	47	6
24	61	12
48	74	19

Example 2: Batch experiments using immobilized enzymes

[0019]

(a) The carriers provided by Membrana GmbH Obernburg under the name ACCUREL MP1001 have a size of 400-1000 μ m. Before the immobilization carrier particles with a size of 1000 μ m are selected by sieving.

For the immobilization, 500 mg of ACCUREL MP1001 in 1.7 ml of ethanol and 100 mg of lipase powder, dissolved in 2.5 ml of potassium phosphate buffer (KH₂PO₄, pH 7, 20 mM), are mixed and shaken overnight in a
35 shaker at room temperature. The immobilized lipase is collected by filtration, washed three times with the same buffer and dried at room temperature for a few hours. The immobilized enzyme is stored at 4 °C until use.

The amount of protein immobilized is determined using a modified Lowry method.

(b) 10 ml of tert.-butyl methyl ether, 100 μ l of water, 20 mg of immobilized enzyme (13 % enzyme / 87 % of ACCUREL carrier) [the lipases are part of a screening kit provided by Roche Diagnostics GmbH (Mannheim, Germany) called "Chirazyme"] and 160 mg of TMHQ-DA are added into a vessel. The headspace of the vessel is flushed with nitrogen. The vessel is placed into an incubator at 33°C and stirred at 700 rpm to ensure good mixing. To take samples, the vessels are opened, 500 μ l are taken out, the headspace is flushed with nitrogen and the vessel is closed again. The sample is then diluted to a substrate or product concentration of 0.5-1.0 wt % and analyzed by
45 GC.

Table 2:

Conversion of pure TMHQ-DA using chirazymes after 96 h (E/S = 1/8, C _{TMHQ-DA} = 0.019 g/g)		
lipase	Source	conversion X [%]
CAL (B)	<i>Candida antarctica</i> (fraction B)	70.0
CRL	<i>Candida rugosa</i>	65.0
CRL (pure)	<i>Candida rugosa</i> (purified)	87.0
CAL (A)	<i>Candida antarctica</i> (fraction A)	0.7

Table 2: (continued)

Conversion of pure TMHQ-DA using chirazymes after 96 h ($E/S = 1/8$, $C_{\text{TMHQ-DA}} = 0.019 \text{ g/g}$)		
lipase	Source	conversion X [%]
PSL	<i>Pseudomonas spec.</i>	96.1
HPL	Hog pancreas	5.0
TLL	<i>Thermomyces lanuginosus</i>	100.0
MML	<i>Mucor mihei</i>	18.0
ASL	<i>Alcaligenes spec.</i>	24.0
HL1 (esterase)	Hog liver (fraction 1)	0.2

[0020] Use of crude TMHQ-DA (see Example 1) results in a relative conversion rate in the range of from 95 % to 100 % as compared to use of pure TMHQ-DA.

Table 3:

Comparison between immobilized PSL and TLL ($E/S = 1/2$, $C_{\text{TMHQ-DA}} = 0.014 \text{ g/g}$, temperature: 50 °C, other conditions see above, selectivity > 99.5 % for both enzymes)		
	conversion X [%]	
time [h]	PSL	TLL
6	10	93
12	20	99
24	37	99

Example 3: Continuous enzymatic saponification in a fixed-bed reactor

[0021] Continuous saponification of TMHQ-DA to TMHQ-1-MA is carried out in a fixed-bed reactor [900 mg TLL immobilized on ACCUREL MP1001; height of bed: 73 mm; diameter of bed: 12.0 mm; bed density: 0.11 g/ml; bed volume: 8.1 ml; carrier diameter: 0.7 mm] at 40°C, substrate flow: TMHQ-DA in a concentration of 0.01 g/g in water-saturated tert.-butyl methyl ether; mass flow of tert.-butyl methyl ether = 0.080 mg/min; and mass flow of TMHQ-DA = 0.85 g/d.

The immobilized TLL is stable and active for at least 224 hours; the selectivity of the TLL in the saponification of TMHQ-DA to TMHQ-1-MA is almost 100% at 100% conversion; and even at long residence times or when feeding a TMHQ-DA solution having a low concentration, saponification of TMHQ-1-MA to TMHQ does practically not occur.

Claims

1. A process for effecting a highly selective conversion of trimethylhydroquinone diacetate into trimethylhydroquinone-1-monoacetate which comprises subjecting the trimethylhydroquinone diacetate to an enzymatic monosaponification by means of a lipase.
2. A process as claimed in claim 1 wherein the lipase is immobilized on a solid carrier material.
3. A process as claimed in claim 1 wherein the lipase is *Pseudomonas spec.* lipase, in particular *Pseudomonas fluorescens* lipase, or *Thermomyces lanuginosus* lipase.
4. A process as claimed in claim 3 wherein the lipase is *Thermomyces lanuginosus* lipase.
5. A process as claimed in claim 1 wherein the enzymatic monosaponification is carried out in a hydrophobic solvent.
6. A process as claimed in claim 5 wherein tert.-butyl methyl ether is used as the solvent.

7. A process as claimed in claim 2 wherein the enzymatic monosaponification is carried out continuously.
8. A process as claimed in any one of claims 1 to 14 which further comprises converting the resulting trimethylhydroquinone-1-monoacetate into (all-*rac*)- α -tocopherol or its acetate by reaction with isophytol or an equivalent thereof, either directly or followed, if desired, by deacetylation.
9. A process as claimed in claim 1 wherein the reactions are carried out under pressure.
10. A process for the production of (all-*rac*)- α -tocopherol or its acetate which process comprises conversion of trimethylhydroquinone diacetate into trimethylhydroquinone-1-monoacetate by means of a lipase and condensation of the resulting trimethylhydroquinone-1-monoacetate with isophytol or an equivalent thereof, optionally followed by deacetylation.



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EUROPEAN SEARCH REPORT

Application Number
EP 02 00 3627

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Y	<p>DATABASE CA 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; PARMAR, V. S. ET AL: "Regioselective hydrolysis of polyacetoxo aromatic ketones with lipases in organic solvents" retrieved from STN Database accession no. 118:80594 XP002201206 * abstract * & INDIAN J. CHEM., SECT. B (1992), 31B(12), 925-9 ,</p>	1-10	C12P7/62 C12P17/06
Y	<p>PATENT ABSTRACTS OF JAPAN vol. 1996, no. 09, 30 September 1996 (1996-09-30) & JP 08 119958 A (KURARAY CO LTD), 14 May 1996 (1996-05-14) * abstract *</p>	1-10	
Y,D	<p>CIUFFREDA P. ET AL.: "Regioselective Hydrolysis of Diacetoxynaphthalenes Catalyzed by Pseudomonas sp. Lipase in an Organic Solvent." TETRAHEDRON, vol. 56, 2000, pages 317-321, XP002201205 * page 318 *</p>	1-10	<p>TECHNICAL FIELDS SEARCHED (Int.Cl.7) C12P</p>
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 5 June 2002	Examiner Douschan, K
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p>		<p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : member of the same patent family, corresponding document</p>	

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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For more details about this annex ; see Official Journal of the European Patent Office, No. 12/82